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CHEMICAL COMPOSITION OF BLOOD OF SMALLMOUTH BASS

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RESEARCH REPORT 57

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Eddie Wayne Shell
Fishery Research Biologist

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UNITED STATES DEPARTMENT OF THE INTERIOR

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FISH AND WILDLIFE SERVICE

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BUREAU OF SPORT FISHERIES AND WILDLIFE

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ABSTRACT

Concentrations of 16 organic and inorganic components of the blood and serum of the smallmouth bass (*Micropterus dolomieui*) were measured every 3 to 4 weeks from June 24 to November 18, 1958. Components measured included proteins, nonprotein nitrogen fractions, phosphorus fractions, electrolytes, and cholesterol in serum; and glucose, creatinine, and iron in blood.

Concentrations of 13 of the 16 components displayed systematic rather than random variation and appeared to be cyclic when plotted against time. Levels of several of the components decreased in successive samples during the early part of the experimental period only to reverse this trend and increase throughout the remainder of the period. Other components displayed inverse behavior to this scheme.

Three periods of different catabolic-anabolic balance have been proposed as being responsible for the cyclic nature of the concentration curves. Available evidence suggests that endocrine relations are responsible for changes noted in blood composition. A scheme of hormone-hormone antagonism is proposed, which would account for the observed results.

CHEMICAL COMPOSITION OF BLOOD OF SMALLMOUTH BASS

By Eddie Wayne Shell, *Fishery Research Biologist*

Cornell University, Ithaca, N.Y.

No problem is more fundamental to understanding total metabolism than is the relation of composition to function in the blood of animals. No single component or part of an animal is more closely associated with total metabolism than is blood. Every organ, every tissue, every cell communicates with every other organ, tissue, and cell by this fluid "party line." The composition of the blood at any one time reflects the state of function of every organ, tissue, and cell at that time. If the composition of blood and the relation of composition to function are known, then it would be possible to determine the state of function at any time by measuring composition. Such information could also be used to predict trends in function.

Clinical diagnosis used so effectively by modern medicine is dependent on information on the composition of blood and the relation of its composition to function. Many specific diseases, whether caused by bacteria or metabolic dysfunction, are characterized by specific changes in the composition of the blood of the animal. Clinical diagnosis depends on the comparison of the blood composition of the animal with a suspected disease with the blood composition of healthy animals of the same species. Obviously, information as to the levels of the various components in the blood of healthy animals and of animals known to have a specific disease must be available.

Information on the blood composition of healthy fish or fish with specific diseases is very scarce. With such information kidney disease, pancreatic necrosis, or other diseases in populations of hatchery fish might be diagnosed before the epidemic stage is reached. Vitamin deficiencies or other nutritional diseases could be detected much earlier than is possible when decreasing growth rates or increasing mortality rates are used as a basis for diagnosis. This project was initiated to provide information on the composition of the blood of normal or apparently healthy fish.

Specifically, this project was initiated to determine the levels of 16 noncellular components in the blood of the smallmouth bass (*Micropterus dolomieui* Lacépède). These components were to be measured periodically over approximately a 6-month period to determine average values, or if the levels changed significantly in this period, determine the extent of change and attempt to relate change in composition to function in the cyclic life patterns of this fish.

This investigation was conducted during the period June through November 1958, by the author while a student at

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Presented to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The author is presently at Auburn University, Auburn, Alabama.

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Cornell University. The laboratory work was conducted at the Cornell University experimental fish hatchery. Smallmouth bass used in the investigation were obtained from a creek adjacent to the campus.

Dr. Arthur M. Phillips, Jr., Associate Professor of Fishery Biology, Cornell University, proposed this study and made many helpful suggestions throughout its course. Dr. Harry W. Seeley, Jr., Professor of Bacteriology, and Dr. Walter L. Nelson, Professor of Biochemistry, reviewed the manuscript.

COLLECTION AND TREATMENT OF SAMPLES

EXPERIMENTAL ANIMALS

Difficulties in maintaining experimental animals under laboratory conditions made the use of a natural population almost mandatory. The bass used in these experiments were taken from Fall Creek, a small creek which receives overflow waters from Lake Como, a few miles southeast of Dresserville, Cayuga County, New York. From Lake Como the creek flows southeastward until it enters Tompkins County, thence to Ithaca, N.Y., where it empties into Cayuga Lake. Fish used in these experiments were taken from a section limited on the north by Malloryville, N.Y., and on the south by a dam at the north end of Beebe Lake on the campus of Cornell University. This section is generally coincident with the area of acceptable bass habitat in Fall Creek.

The fish are more or less evenly distributed from May until September throughout the section described. During the latter part of September or early October, when the water temperature begins falling, the fish tend to move into a few restricted areas of this section where they spend the winter months in a quiescent state. These areas are characterized by bottoms strewn with large flat rocks. This migration or movement to wintering quarters begins when water temperatures are around 50° to 55° F., in late September or early October, and is completed by the end of October when temperatures range from 45° to 50° F. The quiescent state begins when the water temperatures fall below approximately 45°. Throughout the winter and until the temperature begins to rise in the spring, the fish remain underneath the rocks. Apparently they will take food during this period but do not actively seek it.

The population of fish used in these experiments is characterized by the small average size of the individuals. Fish 8 to 10 inches in length comprise the bulk of the population. Only 1 fish as large as 14 inches was taken during the experimental period.

Distribution of the fish in the stream during most of the experimental period made the use of seines, traps, and electrofishing gear impractical. All fish used in these experiments, until the beginning of the quiescent state, were taken by angling with spinning tackle. Once a fish became hooked he was forced to hand as quickly as possible to avoid tearing out the hooks. A 500-volt, variable voltage, direct-current generator with the voltage regulator set for 450 volts was used to take quiescent fish under the rocks. The shocking or electrofishing apparatus was constructed so that the positive electrode was built into a dip net and the negative electrode was attached to a sled-shaped float.

COLLECTING BLOOD SAMPLES

Apparatus

The small kit shown in figure 1 has proved satisfactory in collecting blood in

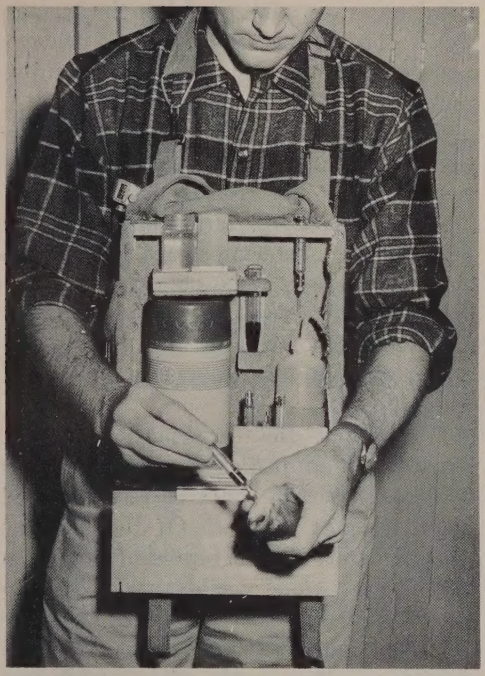

FIGURE 1.—Kit for collecting blood in the field.

the field. It consists of a wooden frame, a solid floor, a pressed-wood panel back, and a cover of canvas panels. The front is closed by a canvas panel that can be buckled to a hinged plywood panel covering the lower half of the kit. This plywood panel protects needles and syringe parts held in a draining and drying rack secured to the floor of the kit. Also attached to the floor are two slotted strips which receive the rolled edge of the bottom of a wide-mouthed vacuum bottle, 7¼ inches high and 4 inches in diameter. A small wooden rack to hold a centrifuge tube while it is being filled with blood is attached to the back of the kit. Also attached to the back is a small receptacle for holding the wash and rinse bottles of distilled water. An assembled syringe is kept in readiness in a hole in the top of the kit. A plastic bottle for reserve distilled water is held by a rubber band in the corner of the kit. In the field, the kit is carried with the aid of a canvas strap around one's neck. A large flat piece of foam rubber sewn to the strap eased support of the weight.

The vacuum bottle holds empty centrifuge tubes as well as tubes of blood in a cylindrical wire rack. The wire rack, with a piece of sponge serving as the bottom of the cylinder, holds the tubes away from the glass lining and also prevents excessive stirring of the ice and water in the vacuum bottle.

Syringes of 2-milliliter capacity with a "needle-lock" were used throughout this experiment. The syringes were large enough to hold the blood from the larger fish, and yet were small enough to be easily manipulated with one hand. Hypodermic needles ¾ inch in length and of No. 20 gage were found to be most suitable for collecting blood from the smallmouth bass. A needle of this length was needed to reach the heart in larger fish and was not unwieldy when used on smaller fish. The 20-gage needle was more practical in the prevention of hemolysis and clotting and was also much easier to keep clean.

Like other fish blood, smallmouth bass blood is highly coagulable. Small clots usually formed within about one-half minute after blood had been drawn. Clotting is of little consequence when the blood is to be used for serum; however, it was difficult to draw blood and transfer it into tubes treated with anticoagulant before clotting ruined the sample for whole blood determinations. This was overcome by preparing a 25-percent solution of potassium oxalate, drawing some of this solution into the syringe and forcing it out again. After forcing out the oxalate, air was drawn in and forcibly expelled several times. After this treatment the walls of the syringe remained moist with the anticoagulant, and blood drawn into the syringe mixed with the anticoagulant as it entered.

Procedure

Blood was collected by cardiac puncture. The needle was inserted at a 90° angle to the axis of the fish, approximately one-half inch behind the apex of the V formed by the isthmus and opercles (fig. 1). The needle point was pushed into and through the chest cavity until slight resistance was encountered. At this time suction was applied by the syringe and the needle slowly withdrawn. As the opening of the needle being withdrawn passed through a heart chamber, blood was drawn into the syringe. Best results were obtained by a slow, gentle insertion. Apparently, a needle of the size used can cause surgical shock severe enough to interrupt the beat of the heart if carelessly inserted.

The blood of a fish was collected so long as it could be easily drawn. It was usually possible to collect at least 0.5 ml. of blood, and up to 2.5 ml. from the larger fish; however, very few fish were large enough to supply 2.5 ml. of blood. The fish sampled averaged approximately 7 inches and the amount of blood collected averaged 0.75 ml. per fish. The blood was gently expelled from the syringe into a 12-ml. conical centrifuge tube. Because of the size of the needle used, blood could be forced through it into the tube without causing hemolysis.

During the sampling, blood was transferred from the syringes into the tubes until 4 to 5 ml. of blood had been collected, at which time the tube was transferred to a wire rack in a wide-mouthed vacuum bottle containing crushed ice. After about 3 hours of collecting, tubes of blood were placed in a refrigerator and held there until the serum was separated.

Both syringe and needle were washed after use by drawing distilled water into them and forcibly expelling it. This procedure was repeated until they had been washed three times from each of two distilled water containers. After the syringe was washed, it was separated into its component parts and placed in a draining rack.

For the entire series of 16 determinations, approximately 23 ml. of whole blood were needed. Of this, about 20 ml. of blood were used for serum and 3 ml. for whole-blood determinations. Enough blood could usually be obtained from 30 fish to satisfy these requirements. From 4 to 10 hours of fishing were required to collect enough fish to supply 23 ml. of whole blood.

Throughout the experimental period fish were fin clipped at the time blood was taken. These fish could be recognized if taken in later catches. Of the total of approximately 350 fish represented in these experiments, blood was drawn more than once from only 3 fish. Fish taken by electric shock and held in concrete pools were moved to other pools after blood was drawn from them.

Sampling schedule

Blood samples were taken according to the following schedule:

Sample I	June 24, 1958
Sample II	July 5, 1958
Sample III	July 24, 1958
Sample IV	August 2, 1958
Sample V	August 18, 1958
Sample VI	August 27, 1958
Sample VII	September 29, 1958
Sample VIII	October 7, 1958
Sample IX	October 31, 1958
Sample X	November 18, 1958

A total of 22 fish (sample X) was collected by electric shock on November 11, 1958, and removed to a small concrete pool at the Cornell University fish hatchery. Pieces of tile pipe were added to the pool to provide cover for the fish in their quiescent state. Since the water temperature in the pool was very similar to the temperature of the normal habitat, no significant error in blood chemistry was introduced

as a result of moving the fish from creek to pool, and values obtained from samples taken from those fish were reliable estimates of changes normally taking place in the blood of fish in a quiescent state in the stream. A blood sample was taken from these fish on November 18.

Additional information on sampling dates, numbers of fish in the samples, and water temperatures during the sampling periods is included in table 1.

TREATMENT OF BLOOD

Blood collected for serum determinations was stored in heavy-walled, 12-ml. conical centrifuge tubes until clots expressed most of the serum. The blood of the smallmouth bass coagulates very readily but clots are very loose. Centrifugation soon after clotting resulted in very poor yields of serum. Also, if centrifugation was carried out soon after clotting, the serum was very difficult to pick up, even with a micropipette, without stirring up cells and transferring them with the serum. To overcome this problem, coagulation was allowed to continue for 12 to 14 hours after the blood was collected. After this period of time, shrinking of the clots had expressed most of the serum so that centrifugation at approximately 2,500 revolutions per minute produced a maximum yield of serum that could be transferred without disturbing the red cells.

A micropipette with a continuous siphon suction apparatus was used to remove the clear serum into a clean, dry centrifuge tube. The serum was held in a refrigerator until determinations were begun. All serum from a given blood sample period was pooled. Since only a small amount of blood could be taken from individual fish, there was little opportunity to get enough material to make determinations on blood or serum of individual fish. By pooling serum from all fish, rather than having a determination of one component from one or two fish and another component from still another fish, it is possible to get a measurement of each of the 16 components from each fish. This in effect gives us a measurement of a component in approximately 30 fish. There was no evidence of hemolysis in any of the samples. The serum was clear, but with a moderate golden-yellowish cast. Blood collected for whole-blood determinations, treated with an anticoagulant, was held in 12-ml. conical centrifuge tubes under refrigeration until time of analysis.

CHEMICAL DETERMINATIONS

Whole blood from the fish sampled in this experiment was somewhat difficult to handle, even with the use of an anticoagulant. Fibrin clots formed readily unless care was taken in introducing the pipettes or in remixing the plasma and red cells after the blood had been setting in the refrigerator for a few hours. This tendency to form fibrin clots very readily made the use of whole blood or plasma somewhat difficult. For this reason 13 of the 16 determinations were made on serum. Only glucose, iron, and creatinine were determined in whole blood.

The scheme of determinations was designed to measure the more labile components as soon as possible. Protein-free filtrates were prepared when practical and stored in screwcap tubes until analyzed. In general, all 16 components could be determined in a 96-hour period after the blood was collected.

All determinations were made with the use of a Lumetron photo-electric colorimeter, employing methods recommended in the manual of the manufacturer.

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BLOOD COMPONENT LEVELS

When this study was undertaken the main objective was to determine normal values for some 16 components in the blood and serum of the smallmouth bass. This information was to be used in a comparative study, or a study comparing the blood composition of several species of fish. It was anticipated that a comparative study of blood composition might provide evidence of the relationship between composition and function in fish, or that habitat requirements (whether salt water, fresh water, and so on), food habits (whether piscivorous, insectivorous, and so on), activity, and other characteristics of a species, might be reflected in blood composition.

Information concerning the blood composition of the normal fish should also be valuable to the fish pathologist. With a series of normal values at hand, it should be possible to describe abnormal, blood-composition patterns characteristic of specific pathological conditions. Characterizations using blood composition should be useful in diagnosing or even predicting disease outbreaks in fish. Information of this type is singularly unavailable to the fishery worker at the present time.

It was anticipated in carrying out determinations periodically that a range of values could be obtained that were normally distributed about a mean or average value; however, it was apparent after the first few series of determinations that the anticipated objective would not be realized. All of the components determined were displaying systematic rather than random variation. Serum total protein and serum globulin levels dropped steadily throughout the first 8 weeks of the experimental period (table 1). During this same interval, serum sodium and potassium increased steadily. All other com-

TABLE 1.—*Concentrations of 16 noncellular components in the blood and serum of the smallmouth bass, measured periodically from June 24 to November 18, 1958*

Item	Samples[1] taken by angling									Sample taken by electro-shock
	I	II	III	IV	V	VI	VII	VIII	IX	X[2]
Date blood sample taken	June 24	July 5	July 24	Aug. 2	Aug. 18	Aug. 27	Sept. 29	Oct. 7	Oct. 31	Nov. 18
Number fish in sample	28	26	41	31	30	28	32	31	4	22
Water temperature (F.)	67°	74°	70°	68°	68°	60°	56°	51°	46°	50°
Blood:										
Creatinine (mg%)	0.5	0.8	1.0	0.7	0.5	0.5	0.5	0.6	----------	0.9
Glucose (mg%)	58	58	63	90	68	85	94	100	----------	88
Iron (mg%)	14	17	12	19	18	20	15	17	----------	19
Serum:										
Protein (gm%)	2.6	2.3	2.2	1.9	1.8	2.3	2.4	2.7	3.3	4.5
Albumin (gm%)	1.2	1.2	1.3	1.1	1.1	1.2	0.9	1.4	----------	1.8
Globulin (gm%)	1.4	1.1	0.9	0.8	0.7	1.0	1.5	2.3	----------	2.7
Albumin-globulin ratio	.86	1.09	1.44	1.38	1.57	1.20	.61	.59	----------	.67
Nonprotein nitrogen (mg%)	30.0	36.1	38.4	30.5	28.2	28.6	28.6	28.2	55.9	59.4
Uric acid (mg%)	4.9	3.8	6.0	3.8	6.4	5.4	4.9	3.8	----------	----------
Cholesterol (mg%)	----------	145	145	145	65	176	346	304	----------	300
Calcium (mg%)	----------	12.8	13.8	11.4	12.3	10.2	10.5	12.0	13.2	19.5
Chloride (meq/l)	123	102	111	116	116	128	132	116	127	110
Phosphorus:										
Total (mg%)	89.6	89.3	70.9	61.7	71.4	74.6	76.5	67.5	----------	75.3
Phosphorus, lipid (mg%)	49.3	51.1	45.9	35.2	46.6	46.9	53.0	51.0	----------	61.5
Phosphorus, inorganic (mg%)	16.0	15.0	12.5	12.8	17.3	12.2	9.1	9.4	8.8	9.2
Potassium (mg%)	9.9	6.4	9.0	12.8	17.2	15.0	10.8	8.0	----------	9.0
Sodium (meq/l)	128	120	128	136	148	140	140	140	----------	140

[1] Blood sample removed at time of capture.
[2] Taken on November 11 and held in a concrete pool until the blood sample was taken on November 18.

ponents showed either increasing or decreasing trends, such as these noted.

It became obvious by the end of the experimental period that a normal blood pattern in the smallmouth bass could not be described without including time of sampling in the description. The pattern that was considered normal on July 24 was not the same pattern recorded on August 18 or September 29. It was also obvious that values obtained in these experiments could not be used in comparative studies. The use of a range to describe extreme values of systematic variation is statistically unacceptable, as is calculation of an arithmetical mean from these values. Once this systematic or cyclic variation was noted, the most desirable objective then became an attempt to ascertain the factor or factors responsible for these changes, or an investigation of the metabolism of fish as it is reflected by the blood of the smallmouth bass.

Information on the blood of fish is meager; information on fish metabolism is almost nonexistent. Most of the work published to date consists of measurement data. Data in which concentrations of various components have been measured are readily available, but data in which concentrations of components have been related to metabolism are lacking. Where there is such a lack of data in the literature with which to interpret experimental results, a worker must of necessity weave his findings with the threads of data available on fish, and then attempt to fill in the gaps with information on mammalian metabolism.

Although all components of the blood of the smallmouth bass studied in these experiments are closely related physiologically, a unified presentation of results and discussion would be very difficult. For this reason individual components or groups of closely related components will be discussed first and then in a general discussion the separate parts will be integrated.

SERUM PROTEINS

Information regarding the serum proteins of fish has been slow in accumulating, and that available is limited in its usefulness by the diversity of conditions, species of fish, and analytical methods with which it was obtained. Most of the work on serum proteins has been limited to the comparative approach, i.e., comparing the serum proteins of one species with those of another. Few experiments have been conducted with the aim of relating serum proteins to their function in the life of the fish; however, several papers on the serum and plasma proteins of fish should be mentioned. Lepkovsky (1929) studies the distribution of serum and plasma proteins in several species of teleosts and one elasmobranch. The distribution of blood proteins in the carp and brown trout has been reported by Field et al. (1943). Deutsch and Goodloe, using electrophoresis, in 1945 reported results of studies on the plasma and serum proteins of fish. Deutsch and McShan (1949) conducted electrophoretic studies on the blood of 17 species of fish, and reported differences of taxonomic importance among many of the species. Phillips et al. (1957) reported results of studies on the serum proteins of brook and brown trout. Flemming (1958) has probably made the most exhaustive study of the serum proteins of fish. Using the electrophoretic method, he studied the serum proteins of the carp. He recorded the pattern of the different protein fractions in carp several times during the year, in starved carp, in carp after a winter in ponds, and in carp infected naturally and artificially with carp dropsy or ascites.

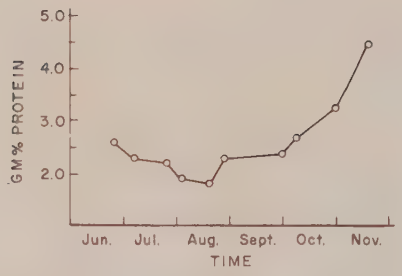

FIGURE 2.—Total protein concentrations in the serum of smallmouth bass, determined periodically from June 24 to November 18, 1958.

Serum total protein

Serum protein is a mixture of two proteins, albumin and globulin. These two molecules vary greatly in their physical, chemical, and physiological properties, but together in the blood they are active in regulating the osmotic pressure and, therefore, the water balance of the organism. They transport nutrient materials needed by the anabolic machinery at the cellular level, and mediate the transfer of catabolic products to the sites of excretion. They transport hormones to chemoreceptive centers and perform logistical activities in the defense against bacterial invaders.

In these experiments the concentration of total serum protein has been determined in the serum of the smallmouth bass at intervals from the end of their spawning season in June until the beginning of their quiescent state in November. The results of these determinations are given in table 1, and are shown graphically in figure 2, where concentration in grams of protein per hundred milliliters (gm%) of serum is plotted against time. As is evident from the table and the graph, serum total protein of the smallmouth bass varies considerably with respect to time. From the June 24 level of 2.6 gm% the concentration decreased gradually until it reached a level of 1.8 gm% on August 18. The concentration then increased throughout the remainder of the experimental period, reaching a level of 4.5 gm% on November 18.

Two observations are of interest in these results:

(1) The cyclic nature of the concentration curve is intriguing. If we accept these values as being reliable estimates of changes taking place in the blood of these fish, then we must suppose that the metabolic patterns which maintain serum protein concentrations are cyclic in nature also.

(2) The extent of variation in the serum protein levels is also interesting. In these experiments a decrease of 30 percent in concentration over an 8-week period was followed by an increase of 150 percent in a 12-week period. In higher vertebrates, changes of this magnitude in serum proteins are recorded only after severe trauma, malignancy, or diseases of the kidney. In the smallmouth bass under the conditions of these experiments, such changes apparently are normal.

The possible role of temperature as cause for the cyclic nature of these data should be mentioned at this point. It is possible that the change noted in the concentration curve of serum globulin between June 24 and August 18 was a result of some physiological state induced by rising water temperatures and that the gradually rising concentration curve of the period August 18 to September 29 simply reflected a cooling trend in water temperature. Temperature data collected during each sampling trip (table 1) does not support this "temperature induced" hypothesis. Only in one interval, June 24 to July 5, did a rise in water temperature coincide with a drop in serum globulin levels to support this hypothesis. From June 24 to July 5, the temperature increased from 67° to 74° F. This 7° rise in temperature was accompanied by a decrease in the serum globulin concentration

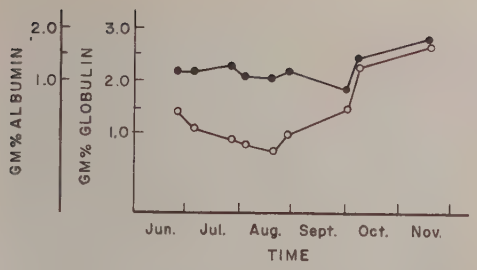

FIGURE 3.—Albumin (solid circles) and globulin (open circles) concentrations in the serum of smallmouth bass, determined periodically from June 24 to November 18, 1958. (Note that scales on the ordinate axes are different.)

from 1.4 gm% to 1.1 gm%; however, the next two decreases (0.9 gm% and 0.8 gm%) are accompanied by two decreases in water temperature (70° and 68° F.). It appears from these data that changes in water temperatures were not responsible for the cyclic changes in the serum globulin concentrations.

Serum globulin

Serum globulin concentrations measured during the 20-week experimental period are recorded in table 1 and are shown graphically in figure 3. This component of the serum decreased from a level of 1.4 gm% on June 24 to 0.7 gm% on August 18. This period of decline was then followed by an increase in concentration to 2.7 gm% on November 18. By comparing these results and the globulin curve with that of total serum protein (fig. 2), it is readily seen that the cyclic nature of the total protein curve is in reality a reflection of changes taking place in the serum globulin fraction. In fact, almost 90 percent of the decrease in total serum protein was due to the loss of serum globulin. From these results then it seems that the cyclic metabolic pattern proposed as being the cause of the changes in total serum protein was in actuality restricted to a cyclic pattern of serum globulin metabolism. That globulin would contribute the major part of the variation in changes of serum total proteins is not surprising. Miller et al. (1949) has shown the half-life of the globulin molecule to be about 3 days, less than half that of albumin. This indicates a very rapid turnover rate or metabolic rate in globulin. This also indicates a more sensitive metabolism, a metabolism that would quickly reflect changes in the catabolic-anabolic relationship. At this rapid rate of turnover, a slight increase in the catabolic rate without an equal increase in the rate of synthesis should result in a decrease in the net amount of globulin. Also, a decrease in energy supply for synthesis and the resulting lowering of the anabolic rate without a decrease in the catabolic rate would have the same effect. An increase in catabolism and a simultaneous decrease in anabolism would rapidly reduce the net amount of globulin. Any one of these three catabolic-anabolic relationships could explain the cyclic nature of the serum globulin concentration observed in these experiments.

Serum albumin

Serum albumin levels of the smallmouth bass changed only slightly during the period June 24 to September 29. Data presented in table 1 and figure 3 show that albumin decreased only 0.1 gm% during the same period that serum globulin levels fell 0.7 gm%. These results are not unexpected. If the evidence concerning the comparative half-lives of the albumin and globulin molecules be applied here, changes in the concentration of serum albumin would be expected only after serum globulin had shown marked changes. However, the explanation does not appear to be quite as simple as has been presented thus far. As seen in figure 3, after September 29 the albumin and globulin curves show identical patterns of

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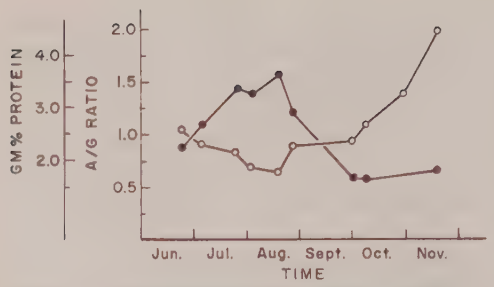

FIGURE 4.—Albumin-globulin ratios (solid circles) and total protein (closed circles) concentrations in the serum of smallmouth bass, determined periodically from June 24 to November 18, 1958. (Note that the scales on the ordinate axes are different.)

increase. Both fractions increase abruptly from September 29 to October 7, then the curves flatten out together until November 18. These curves suggest that some mechanism maintains serum albumin levels when serum globulin levels fall, but that factors favoring a net increase of serum globulin also favor an increase in albumin. Elucidation of this mechanism is not possible with these data.

Albumin-globulin ratio

The albumin-globulin ratios (A/G) during the experimental period showed an inverse relation with serum globulin and serum total protein (fig. 4). This is not unexpected, since serum albumin remained relatively constant throughout most of the experimental period while serum globulin changed markedly. In these experiments the A/G ratio increased from a value of 0.86 on June 24 to 1.57 on August 18. The values then decreased until they attained a more or less stable level after September 29 (table 1 and fig. 4).

The A/G ratio is always greater than 1.0 in mammals. Values approaching 1.0 in man are indicative of impairment of kidney or liver function. Results of determinations of the A/G ratio in fish are confusing. Lepkovsky (1929) reported that the A/G ratio of the menhaden ranged from 1.28 to 2.08, the goosefish from 0.17 to 0.54, the dogfish from 0.09 to 0.35, and the bullhead from 0.20 to 0.36. Field et al. (1943) recorded an A/G ratio of 3.57 for the carp and 2.26 for the brown trout. Phillips et al. (1957) reported an A/G ratio of 2.19 in the brook trout and 0.77 for the brown trout. The significantly different A/G ratios recorded by Phillips et al. (1957) and by Field et al. (1943) for the brown trout (2.26 and 0.77) are tentatively explained by Phillips. He noted the presence of an abundance of diplobacilli which are usually associated with kidney disease in the brown trout. Although typical lesions of the disease were not present, an abundance of the organism could have impaired kidney function enough to bring about excessive loss of albumin in the urine. Dombrowski (1954) reported A/G ratios ranging from 0.53 to 1.23 in carp infected with ascites, a disease characterized by an accumulation of serous fluid in the peritoneal cavity.

On the basis of A/G ratios determined in these experiments, it is possible that differences in A/G ratios reported by other workers are a result of a cyclic relationship. For example, the A/G ratio for the smallmouth bass on June 24 was 0.86, but on August 18 it would have been recorded as 1.57, an increase of about 85 percent. Later in the period an A/G ratio of 0.59 was recorded. These results suggest that the A/G ratio is dependent on the time of year it is determined. This cyclic relationship is probably dependent on several factors characteristic of the yearly life cycle of a particular species of fish. Such things as time of sex-product formation, time of spawning, and response to temperature changes, would affect the timing of the cycle and also the amplitude, but due to the cyclic nature of the causes, the cyclic relationship would prevail throughout the life of the individual.

SERUM NONPROTEIN NITROGEN

The concentration of no other fraction or component of the serum of animals is dependent on as many factors as is nonprotein nitrogen. The concentration of this serum fraction is dependent on the digestion of proteins and the absorption of amino acids, the deamination of amino acids, degradation of the nucleoproteins, the efficiency of the excretory system, and the delicate balance between catabolism and anabolism. Nonprotein nitrogen is truly a fluid record of nitrogen metabolism in the animal body, and yet few components of the serum are more constant. This relative constancy of the whole fraction and the metabolic history which its components record have made nonprotein nitrogen extremely important in clinical diagnosis of pathological conditions. For example, urea levels in man above 10 to 15 milligrams per hundred milliliters of serum (mg%) are indicative of chronic and acute nephritis, metallic poisoning, intestinal obstruction, and some infectious diseases (Hawk et al., 1954). The range of the amino acid nitrogen fraction in the blood of the normal man is 5 to 8 mg%. Values higher than this are indicative of leukemia, acute yellow atrophy of the liver, or severe nephritis (Hawk et al., 1954).

The composition of nonprotein nitrogen in the serum of fish is somewhat different than that in man or other vertebrates. Whereas most of the nitrogen in the nonprotein nitrogen fraction of man is in the form of urea, amino acid nitrogen contributes the bulk of the nonprotein nitrogen in fish. The total concentration of nonprotein nitrogen is remarkably similar in man, the rat, and fish.

The nonprotein nitrogen fraction of fish serum has probably been studied more than any other fraction. Comparative physiologists and taxonomists have found fertile ground for their investigations here. Among the papers available, those by Denis (1913), Wilson and Adolph (1917), Field et al. (1943), and Phillips et al. (1957) are the most rewarding. These authors have partitioned the nonprotein nitrogen of several species of fish into its components; however, as with the serum proteins, little information is available concerning the normal or abnormal metabolism of this fraction and its components.

The experimental scheme for the determination of nonprotein nitrogen was the same as that described for serum protein. In this study, total nonprotein nitrogen has not been completely partitioned. Only total nonprotein nitrogen, creatinine, and uric acid have been determined. The results of these determinations are given in table 1, and are graphically presented in figures 5 and 6, where the concentrations in milligrams per hundred milliliters of serum (mg%) are plotted against time.

Total nonprotein nitrogen

The total nonprotein nitrogen fraction rose sharply from the beginning of the experimental period in June until the latter part of July at which time it declined, returning to its original June level about the middle of August (fig. 5). During this period the concentration rose from 30 mg% and fell to about 28 mg%. From

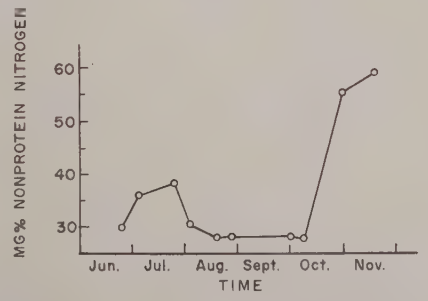

FIGURE 5.—Total nonprotein nitrogen concentrations in the serum of smallmouth bass, determined periodically from June 24 to November 18, 1958.

the middle of August until early October the concentration remained constant; however, between early October and the latter part of October it had risen almost 100 percent, to about 56 mg%. The increase continued into November, where at the end of the experimental period the nonprotein nitrogen concentration was about 59 mg%.

According to Field et al. (1943), the nonprotein nitrogen level in the blood of mammals is chiefly determined by the balance among the rates of protein catabolism, amino acid anabolism, and excretion by the kidneys. This same interrelationship probably exists in fish; although the factors contributing to the balance are somewhat different. In fish, the gills are the main nitrogen excretory site and ammonia is the chief nitrogenous excretory product (Smith, 1929); however, as mentioned previously, the largest fraction of nonprotein nitrogen in the blood of fish is contributed by amino acid nitrogen. This suggests a very different pattern of nitrogen metabolism in the fish. For if ammonia is the chief excretory product, yet is not found in large concentrations in the blood, then deamination of the amino acids must take place at the site of excretion—the gills. Also, the presence of large amounts of amino acid nitrogen in the blood suggests some deficiency in the metabolic machinery of the fish, some defect that makes the utilization of amino acids somewhat inefficient.

The machinery of nonprotein nitrogen metabolism appears to be somewhat different in fish than in mammals, but the total effect, that of keeping the nonprotein nitrogen fraction constant, is comparable. This gives us some basis for probing for the explanation of changes in the nonprotein nitrogen in the serum of smallmouth bass noted in these experiments. The increase in serum nonprotein nitrogen from June 24 to July 24 and the gradual return to the June 24 level of about 28 mg% on August 18 probably reflect an increase in the catabolic rate. Of the three basic factors affecting nonprotein nitrogen levels—anabolism, catabolism, and excretion—a change in the catabolic rate of this natural population of fish seems more likely than a change in either anabolism or excretion. According to this assumption, the catabolic rate increased from June 24 to July 24, pouring an added amount of nonprotein nitrogen into the blood stream. Then the catabolic rate returned to its June 24 level on August 18. It will be recalled that this period of increased nonprotein nitrogen levels corresponds to the period of decreasing serum protein levels. It might seem at first glance that the changes in these two components are cause and effect, or that the decrease in serum protein reflects an increased catabolic rate of this fraction. This increased catabolic rate pours an added amount of nitrogen into the blood stream to be excreted, this added amount of nitrogen appearing as nonprotein. Rather than this simple cause and effect relation, evidence will be presented later in support of the postulation that both an increase in nonprotein nitrogen and a decrease in serum protein are effects of the same cause, and that this mechanism brings about a simultaneous increased catabolic rate and reduced anabolic rate.

The period of relative constancy of the nonprotein nitrogen fraction, August 18 to October 7 (see fig. 5), probably reflects stable metabolic relations, relations depicting a positive nitrogen balance. In this metabolic situation, the nitrogen in the nonprotein nitrogen fraction arises primarily from the turnover of endogenous body protein. It is a situation where anabolism is proceeding at a faster pace than is catabolism. That anabolism is increased is evidenced by the increasing serum protein concentration during this period.

Nonprotein nitrogen increased significantly from October 7 to October 31 and remained at that high level until the end of the experimental period on November 18 (table 1 and fig. 5). During this period the concentration increased from approximately 28 mg% to 59 mg%, an increase of more than 100 percent. At first glance this increase appears to be the result of a substantial increase in protein catabolism, and this might be true except that during this period there was a net increase of 1.8 grams of serum protein per 100 milliliters of serum. If the increase in nonprotein nitrogen resulted from an increase in catabolism, apparently it was a selective catabolism, since the serum proteins were not affected. There is another possible explanation for this rapid increase in nonprotein nitrogen. A decrease in the efficiency of the excretory system, such as might have been induced by the quiescent state, could have caused such a rapid rise. Here a more complete partition of the nonprotein nitrogen would have been valuable in explaining this phenomenon. The possibility also exists that both of the aforementioned causes contributed. A combination of increased tissue catabolism brought about by the fasting condition of the quiescent fish together with a decreased efficiency of excretion could account for the increase in nonprotein nitrogen. Morgulis and Edwards (1924) suggested that this combination might be the cause of the increase in nonprotein nitrogen in fasting persons.

Blood creatinine

Blood creatinine, the anhydride of creatine, is the least variable nitrogenous constituent of the blood of mammals (Hawk et al., 1954). Results reported by Wilson and Adolph (1917), Field et al. (1943), and Phillips et al. (1957), indicate that this is also true in the case of fish. Hawk et al. (1954) reports that

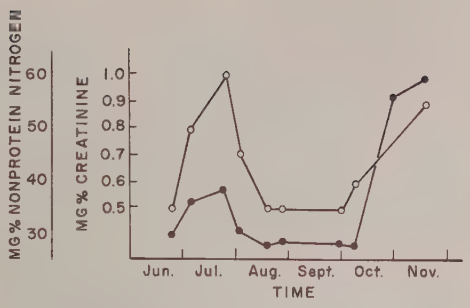

FIGURE 6.—Creatinine (open circles) concentrations in the blood of smallmouth bass, determined periodically from June 24 to November 18, 1958. The serum total nonprotein nitrogen (closed circles) curve from figure 5 is shown for comparative purposes. (Note that the scales on the ordinate axes are different.)

creatinine in mammals is more readily excreted than is either uric acid or urea, and that even a slight increase in blood creatinine is evidence of marked impairment of kidney function. In contrast with ammonia, which is excreted by the gills, creatinine is excreted by the kidney in fish (Smith, 1929).

In these experiments, the blood creatinine concentration of smallmouth bass followed closely the pattern previously described for total nonprotein nitrogen. Although the range of concentration was small, the changes in concentration with respect to time are essentially the same as those noted in nonprotein nitrogen. As can be seen from table 1 and figure 6, there was an increase in blood creatinine during the early part of the experimental period, followed by a period of relative constancy, which was followed by a period of increase.

If results obtained in these experiments were examined, using information from mammalian physiology, the two periods of increased creatinine concentration, June 24 to August 18 and October 7 to November 18, would be interpreted as indicating marked impairment of kidney function (Hawk et al., 1954). Appar-

ently, creatinine clearance in the mammalian kidney is so efficient that increased production of creatinine from creatine normally cannot be detected in the blood, so that any increase in creatinine concentration means an impairment of kidney function. Information of this nature is not available for fish; however, it is not likely that kidney dysfunction was the precipitating cause of the increases in creatinine concentration noted in this natural population of smallmouth bass. When interpreted in the light of changes in other blood and serum components, these changes appear to indicate a rather fixed capacity for creatinine clearance, so that increases of creatinine production would be reflected by changes in blood concentration.

According to Fruton and Simmonds (1958), the creatinine excreted in the urine of mammals is correlated with the fate of the muscle creatine, and in conditions characterized by the breakdown of muscle tissues, the excretion of this compound rises markedly. If the assumption is correct that the fish kidney has a rather fixed capacity for creatinine clearance, then an increase in muscle-tissue catabolism would be indicated by an increase in blood creatinine. Interpreted in this light, the two periods of increased creatinine concentration reflect increases in tissue catabolism. This interpretation agrees with the one arrived at previously for total nonprotein nitrogen.

Uric acid

Uric acid has been determined in the blood and serum of fish by several workers, Denis (1913), Vars (1934), Field et al. (1943), Dombrowski (1953), and Phillips et al. (1957). Their results encompass several species of fish, but show very few differences with respect to the species. In fact, different workers report significantly different concentrations from the same species. This is not an unusual situation. Even in homeostatic animals, uric acid determinations are not useful indicators of purine metabolism, either normal or pathological (Hawk et al., 1954).

Uric acid concentrations in the serum of the smallmouth bass as determined in these experiments are recorded in table 1. These results seem to bear no resemblance to patterns observed in total nonprotein nitrogen and creatinine. The tendency for high and low values to alternate during the experimental period is interesting, but with the information available, explanation is difficult.

SERUM PHOSPHORUS

Information concerning phosphorus in the blood and serum of fish is scarce. Only four papers were located in which blood or serum phosphorus or any of its fractions had been determined. McCay (1929) has made the most comprehensive study to date of phosphorus in the blood of fish. He determined partitioned phosphorus concentrations in the whole blood, in the red cells, and in the serum of catfish. In a later paper, McCay (1931) compared the partitioned phosphorus concentrations in the whole blood, in the red cells, and in the plasma of the pike, carp, and bullhead. Field et al. (1943) included serum inorganic phosphorus and serum total phosphorus in the determination of inorganic constituents of carp blood. These same fractions were also determined by Phillips et al. (1956) in the brown trout. From the work of these authors, apparently the serum of fish contains roughly two times as much phosphorus as the serum of man (Hawk et al., 1954). The serum phosphorus fractions, inorganic phosphorus, organic acid-soluble phosphorus, and lipid phosphorus, also appear in fish serum in approximately two times their concentration in man.

In these experiments three phosphorus fractions were determined. Serum total phosphorus, serum lipid phosphorus, and serum inorganic phosphorus have been determined according to the same experimental scheme as the serum proteins, and other components. The results of these determinations are shown in table 1.

Serum total phosphorus

Results of determinations of serum total phosphorus are given in table 1 and are shown graphically by figure 7. From a very high level of almost 90 mg% on June 24 and July 5 the serum total phosphorus level dropped abruptly, reaching a level of about 60 mg% on August 2. From this low point, the levels increased gradually to about 75 mg% on September 29. This was followed by a sharp drop to about 68 mg% on October 7, and then by an increase to about 75 mg% at the end of the experimental period.

One of the most interesting points in these results is the magnitude of the serum total phosphorus levels. Reported levels in other fish have seldom approached even the lowest values noted in these experiments. McCay (1931), discussing differences in the total phosphorus levels of pike, carp, and bullhead plasma, proposed that the differences might be related to the activity of the three species. If high phosphorus levels were associated with activity, then the smallmouth bass used in these experiments would be expected to have extremely high values. However, work by Phillips et al. (1956) on the blood of brown trout, a very active species, tends to discredit the "activity–high phosphorus level" explanation. He reported an average value of 21.1 mg% for the serum total phosphorus concentration in hatchery brown trout, a value about one-third of those recorded in these experiments.

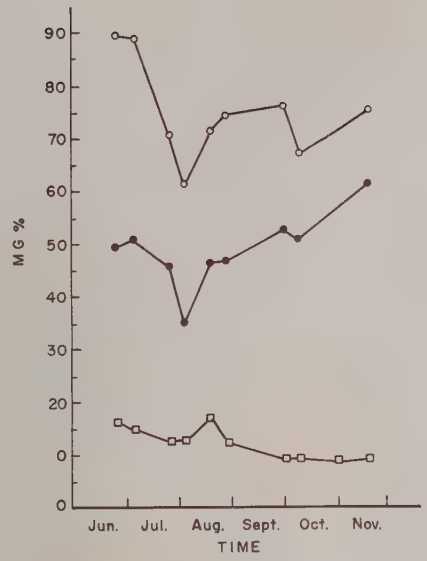

FIGURE 7.—Total phosphorus (open circles), lipid phosphorus (closed circles), and inorganic phosphorus (open squares) concentrations in the serum of smallmouth bass, determined periodically from June 24 to November 18, 1958.

Serum lipid phosphorus

Experimental results of serum lipid phosphorus determinations are reported in table 1 and are shown graphically in figure 7. Lipid phosphorus values reported here are approximately two times as large as those reported by McCay (1929, 1931) in the carp, pike, and bullhead. The part contributed by serum lipid phosphorus to total phosphorus is demonstrated by a comparison of the two concentration curves (fig. 7). Although the magnitude of the changes are different, the direction of change is always the same.

The most interesting result of these determinations of serum lipid phosphorus lies in the similarity of changes occurring in lipid phosphorus and serum protein (see figs. 2 and 7). Both curves dropped to minimum values in August and then rose more or less steadily to their highest values by the end of the experimental pe-

riod. Certain differences are important, however. The lipid phosphorus curve reached its lowest point on August 2, while the serum protein reached its lowest value on August 18. Except for the sharp drop in concentration from July 24 to August 2, the relative magnitude of the decrease in lipid phosphorus was not as great as in serum protein. These results suggest that some mechanism producing a net loss of serum protein, and especially of serum globulin, also produced a net loss in serum lipid phosphorus, and that when the effect of this mechanism was reduced, the two components increased in concentration. These results suggest that serum lipid phosphorus was not equally affected, and that only when the causal mechanism was exerting its greatest influence, between August 2 and August 18, was serum lipid phosphorus markedly reduced. This pattern of behavior in the two serum fractions would be expected if energy for synthesis had been systematically reduced during this period.

Both serum protein and serum lipid phosphorus supposedly require energy in the form of high energy phosphate bonds (adenosine triphosphate, ATP) for synthesis. But the synthesis of the serum protein molecule synthesized by specific attachment of ATP-activated amino acids to a nucleic acid template, as suggested by Dounce (1952), Boorsook (1953) and Fruton and Simmonds (1958), would require a great deal more energy (ATP) than would the synthesis of a lipid phosphorus molecule by the "cytidyl–transferase system," in the manner proposed by Smith et al. (1957). If these assumptions are correct, then a reduced energy or ATP supply brought about by a decrease in the rate of energy production would reduce the amount of energy available for all energy-requiring processes including synthesis. Since the synthesis of serum proteins requires a great deal of energy, a shortage in the supply of energy or ATP would be reflected in a lowered rate of production. Synthesis of lipid phosphorus, which represents a relatively small amount of energy, would be much less affected by a decreased supply of energy. Only after the energy supply became seriously reduced would the synthesis of lipid phosphorus be markedly affected.

An energy shortage could explain the changes noted in serum proteins and lipid phosphorus in these experiments. It would suggest that the energy supply available for synthesis in the smallmouth bass decreased during the period from June 24 to sometime between August 2 and August 18. At this point the supply of energy began to increase, bringing about an increased rate of synthesis. This increased synthetic rate is reflected in the net increase of both serum protein and serum lipid phosphorus, which continued to the end of the experimental period.

Serum inorganic phosphorus

Except for the August 18 blood sample, inorganic phosphorus levels showed a general downward trend throughout the experimental period (table 1 and fig. 7). From a level of 16.0 mg% on June 24, the concentration decreased to 12.8 mg% on July 24. This was followed by a sharp rise to 17.3 mg% on August 18. In the blood sample taken on August 27, the inorganic phosphorus level had fallen to 12.2 mg%. By September 29 the concentration had decreased to about 9 mg%, where it remained until November 18.

The downward trend in concentration levels noted in serum inorganic phosphorus was not observed in any of the other 15 components determined. A systematic reduction in concentration was noted in several serum components (globulin, lipid phosphorus, calcium, et cetera), but in all of these cases there was a reversal of trend during the experimental period. For ex-

ample, serum globulin levels decreased progressively from June 24 to August 18 only to be followed by a progressive increase to the end of the experimental period (fig. 3). These results suggest that the inorganic phosphorus levels in the serum of the smallmouth bass are controlled by a different mechanism than are the levels of other components.

The sharp increase in inorganic phosphorus from August 2 to August 18 corresponds with the period in which globulin levels reached their lowest value, sodium and potassium levels reached their peak, and the energy supply in the smallmouth bass was at its lowest level. When these data are considered along with the discussion in the previous paragraph, it seems possible that serum inorganic phosphorus levels are controlled by a different mechanism than are the other components; that this mechanism is not affected in a similar manner by changes in the metabolism of fish; but that this mechanism may be affected by extreme changes in the anabolic–catabolic balance, such as the condition prevailing between August 2 and August 18 of the experimental period.

SERUM ELECTROLYTES

Electrolytes are distributed in solution throughout all the body fluids, blood lymph, intracellular fluids, digestive juices, and urine. The cations of the electrolytes are the ions of sodium, potassium, calcium, and magnesium, with sodium and potassium predominating. Quantitatively, the chief cation of serum and other extracellular fluids is sodium, while the chief cation of intracellular fluid is potassium. The chief anions of serum and other extracellular fluids are the chloride ion and the bicarbonate ion (West, 1957).

The electrolytes of body fluids have various functions, the most important of which are to contribute a majority of the osmotically active particles, to provide buffer systems and mechanisms for the regulation of pH (acid-base balance), and to give the proper ionic balance for normal neuromuscular irritability and tissue function (West, 1957).

The electrolytes of the animal body are important not only qualitatively but also quantitatively. The distribution of the different electrolytes between intracellular and extracellular fluids is also important. In man the normal serum potassium concentration ranges from 4.0 to 5.4 milliequivalents per liter (meq/l). If this concentration increases to 7 meq/l, characteristic electrocardiographic disturbances are noted. In concentrations above 10 meq/l, the heart may stop in diastole.

The electrolytes of the animal body also perform other important functions. The role of calcium in the clotting of blood and in bone formation is well known. Magnesium is needed for the activation of certain enzymes, such as the phosphatases and transphosphorylases. Sodium and potassium are also necessary in the activation of certain enzymes.

A great deal of information is available regarding the electrolytes of the physiological fluids of fish. Most of this information concerns the role of these ions in osmoregulation. Krogh's work (1939) on the osmotic regulation in aquatic animals is a classical work on this subject. Baldwin (1949) also contributed valuable information on the role of ions in the blood of aquatic organisms. Experiments by Phillips and his group have contributed immensely to information on the role of the calcium ion in osmoregulation (Phillips et al., 1952, 1953, 1954, 1955, 1956, 1957). Compared with this large amount of work on the effect of ions on osmoregulation, little information is available on the role played by the electrolytes in acid-base balance, normal-tissue function, and neuromuscular irritability in the fish.

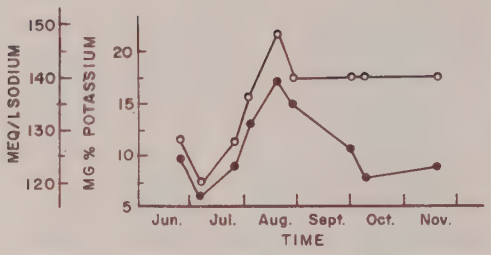

FIGURE 8.—Sodium (open circles) and potassium (closed circles) concentrations in the serum of smallmouth bass, determined periodically from June 24 to November 18, 1958. (Note that the scales on the ordinate axes are different.)

Serum sodium

Serum sodium concentrations, except for an initial decrease, rose markedly throughout the period June 24 to August 18 (table 1 and fig. 8). From a level of 128 meq/l on June 24, the serum sodium level decreased slightly to 120 meq/l on July 5. Following this decline the concentration rose to a maximum value of 148 meq/l on August 18. On August 24, the serum sodium level decreased slightly to 140 meq/l, where it remained until the end of the experimental period.

Serum sodium levels in the normal man are fairly constant, 130–144 meq/l (Hawk et al., 1954). Work by Field et al. (1943) reported a range of 10 meq/l (127–137 meq/l) in the serum sodium levels of the carp, while Phillips et al. (1956) reported a range of 11 meq/l (151–162 meq/l) in the brown trout. In the smallmouth bass, the range of the seasonal variation in the pooled serum sodium values was 28 meq/l (120–148 meq/l). The values of Field et al. and Phillips et al. are not strictly comparable with the values in these experiments, however, since their work was conducted over a shorter period of time.

The homeostatic mechanism for maintaining serum sodium levels seems to be almost as effective in fish as in higher vertebrates. Results of these present experiments suggest, however, that this mechanism in fish is not capable of maintaining a constant level over a period of several months. The cyclic behavior of the regulatory or homeostatic mechanisms in the smallmouth bass has been suggested by evidence in several previous sections of this paper, and seemingly here is another case in evidence. Apparently the needs of the fish change with respect to the amount of the electrolyte sodium, and the homeostatic mechanism seems to be readjusted to these needs.

An interesting aspect of these data is shown by comparing the curves of serum protein (fig. 2) with the serum sodium curve (fig. 8). On August 18, serum protein reached its lowest recorded level and on this same sampling date serum sodium reached its maximum concentration. These two serum components displayed inverse patterns of behavior throughout the period July 5 to August 27. During this period, a decrease in the one component was accompanied by an increase in the other. This relation was disrupted after August 27 and the sodium concentration remained constant throughout the remainder of the experimental period, even though serum protein increased significantly.

Serum potassium

As shown in figure 8, serum potassium concentrations behave in a manner similar to serum sodium. Although the magnitude of changes were different in the two components, the trends in variation were identical from the beginning of the experimental period until August 27. The serum potassium at the beginning of the experimental period was 9.9 mg% (table 1). After a decrease to 6.4 mg%, the concentration increased steadily through four sampling dates until it reached a level of 17.2 mg% on August 18. This rapid increase was followed by a decrease in con-

centration until October 7. At the end of the experiment, the concentration was only slightly lower than the concentration at the beginning of the experiment.

The relation between serum sodium and serum potassium concentrations in these experiments should be mentioned. Changes in the serum sodium and the serum potassium concentrations between consecutive sampling dates are shown in table 2. Also recorded are the ratios of those changes. For example, between June 24 and July 5 the sodium concentration decreased 18 mg%. During the same period, serum potassium decreased 3.5 mg%. Here the decrease in sodium was 18/3.5, or 5.1 times as great as the potassium decrease. Between the sampling dates, July 5 to July 24, sodium increased 18 mg% while serum potassium increased 2.6 mg%. The increase in sodium was 18/2.6, or 6.9 times as great as the increase in potassium. In the next interval, July 24 to August 2, the sodium increase was 19/3.8, or 5 times as great as the potassium increase. As shown in table 2 and suggested by figure 8, any change in sodium concentration, increase or decrease, is accompanied by a similar change in serum potassium, but with approximately one-sixth of the magnitude.

The way in which the information on the relationship of serum sodium and serum potassium has been presented is not meant to imply that a change in the concentration of potassium resulted from a change in the sodium concentration, or that the reverse relationship was true. Although either of these relationships might occur, the ratio employed was simply the easier method, in that ratios calculated in this manner are whole numbers rather than small decimal fractions.

The relation between the levels of serum sodium and serum potassium as presented in table 2 does not explain why these two electrolytes increased in concentration during the period that serum protein decreased. Nor does it explain why the electrolytes reached their maximum concentration on August 18, the sampling date that the serum protein concentration was at its lowest point. However, this relationship does suggest that despite the magnitude of the changes demonstrated, some homeostatic device has been active in maintaining the correct balance between these two important electrolytes, in maintaining a correct sodium–potassium ratio.

TABLE 2.—*Change and ratio of change of serum sodium and potassium concentrations between sampling dates*

Blood sample number	Date of sample	Change in serum concentration (in mg%) of—		Ratio of change
		Sodium	Potassium	
I	June 24, 1958			
		[1] 18	[1] 3.5	5.1:1
II	July 5, 1958			
		18	2.6	6.9:1
III	July 24, 1958			
		19	3.8	5.0:1
IV	August 2, 1958			
		27	4.4	6.1:1
V	August 18, 1958			
		[1] 18	[1] 2.2	8.2:1
VI	August 27, 1958			

[1] Concentration decreased between these 2 sampling dates.

There are two possible explanations for the abrupt rise in the serum sodium and potassium curves (fig. 8) during the period when the serum protein and serum lipid phosphorus concentrations were decreasing. First, in the discussion of the nonprotein nitrogen fraction, an increased catabolic rate was suggested as the reason for the increased nonprotein nitrogen levels from June 24 to August 18. It was also suggested as a partial cause for the decrease in serum protein. If this assumption is correct and the increase in the catabolic rate were great enough to initiate tissue catabolism, potassium would be released into the plasma as a result of cellular disruption. A negative potassium balance associated with abnormal destruction of body tissue in mammals has been discussed by West (1957) and Hawk et al.

(1954). According to this proposition, the increase in serum potassium caused by catabolism of tissue would be accompanied by an increase in serum sodium as a result of activity on the part of the homeostatic device responsible for maintaining the correct sodium-potassium ratio. Once the tissue catabolism ceases, as evidenced by the return to normal of the nonprotein nitrogen curve on August 18 (fig. 5), the potassium levels fall.

The second possible explanation of the rapid increase in serum sodium and potassium concentrations concerns the adjusting of osmotic pressure of the serum or plasma. West (1957) discusses the increased rate of reabsorption of sodium salts in the distal tubules of the kidney as a method whereby the mammalian body can correct conditions in which electrolyte concentration and osmotic pressure of plasma fall below normal. In addition, he notes that this condition is also alleviated by excretion of a more dilute urine, thereby concentrating the plasma somewhat and increasing the osmotic pressure. The situation in the blood of the bass is not exactly comparable with the one that West describes, in that the fish apparently has no electrolyte deficit. It is assumed, however, that as a result of the decrease in serum protein there must be a decrease in osmotic pressure in the bass. It is possible, then, that the increase in serum electrolytes is a compensatory activity on the part of the homeostatic mechanism of the fish to maintain the total number of osmotically active particles in the plasma despite a net loss of serum protein molecules. Increase in the total number of osmotically active particles is possibly brought about by increased active transport of the sodium and potassium ions across the gill membranes into the body (Krogh, 1939). An increase of electrolytes in the plasma could be even more deadly than the loss in osmotic pressure if the different electrolytes were not present in the proper amounts. In this case, even though the concentration of both sodium and potassium have increased, these increases have been maintained in physiological balance (table 2). This was pointed out in the discussion on the sodium–potassium relation.

Serum calcium

Calcium is an essential constituent of all living cells. Its mode of action is not clear, but it appears to play a part in decreasing the permeability of cell membranes. Its effect on neuromuscular mechanisms is shown in higher animals by the development of hyperirritability and tetany as a result of a decline in calcium content of the blood (Hawk et al., 1954). Calcium is apparently necessary in activation of the adenosine triphosphatase activity of myosin, but may uncouple oxidative phosphorylation when present in excess (Fruton and Simmonds, 1958).

In fish, the calcium ion plays an important role in osmoregulation. Phillips et al. (1954) have shown that calcium is absorbed from the water by small, nonfeeding brook trout, and that the absorbed calcium is deposited in body tissues. His group has also shown highly significant differences in the amounts distributed between body parts. Other experiments by this group show that the rate of distribution of calcium to the body parts is a function of the metabolic activity of the trout. They have also shown that the metabolic activity is dependent to some extent on the physical composition of the water. In a later work, Phillips et al. (1957) have shown that the concentration of calcium in the water significantly affects the utilization of calcium from food. More calcium was absorbed by the brook trout from its food in water containing 5.0 parts per million (p.p.m.) calcium than from waters containing 50.0 p.p.m.

Discussing the management implications of calcium in osmoregulation, Phillips et al. (1956) suggested that since energy must be expended by the fish to maintain osmotic equilibrium, the increased metabolic activity on the part of the fish in low-calcium waters could possibly influence the growth of fish. The increased utilization of energy to maintain osmotic equilibrium would leave less energy available for anabolism or growth. In support of this he cites work by Reimers et al. (1955) that showed an apparent positive correlation between calcium content of alpine lake waters and the growth of brook trout.

As is evident from the previous section, there has been a great deal of critical work on the osmoregulatory effect of calcium as well as the effect of changes in calcium concentration on the metabolic rate, but there is little information available on what part this ion plays in metabolism or what changing blood levels mean in terms of changing metabolic patterns. In these experiments, changes in the serum levels of calcium were pronounced (table 1 and fig. 9). Although variation in serum calcium was more erratic, at least during part of the experimental period, it nevertheless demonstrated cyclic tendencies. The calcium concentration during the period July 5 to August 27 was characterized by alternate increases and decreases, but it demonstrated an overall decrease. Following a period of about 4 weeks (August 27 to September 29) in which the concentration remained very nearly the same, the levels began to increase, finally reaching a level of almost 20 mg% at the end of the experimental period.

The behavior of the calcium concentration is very difficult to correlate with behavior of any of the other components. Although it tended to behave generally like serum protein, the relation is not so clear as the relation between protein and

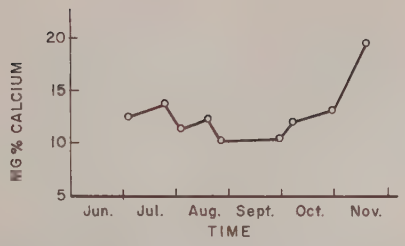

FIGURE 9.—Calcium concentrations in the serum of smallmouth bass, determined periodically from July 5 to November 18, 1958.

sodium or protein and potassium. According to Hawk et al. (1954), there is a reasonably close relation between serum calcium, inorganic phosphate, and protein in man. The relation can be expressed by a multiple-regression equation. This relation does not seem to exist in the smallmouth bass. Although the correlation of serum calcium levels with serum protein levels would probably be significant, the serum inorganic phosphorus levels seem to be totally unrelated to either. Serum calcium levels in these experiments do seem to be cyclic in nature, but with the present state of information on factors affecting the regulation of this electrolyte in the blood, no explanation can be offered for its behavior.

Serum chloride

The chloride ion is intimately involved in several physiological processes of the fish. In addition to being the chief anion of both gastric juice and serum, the chloride ion maintains acid-base balance by its participation in the "chloride shift," and it also contributes a large number of osmotically active particles to the process of osmoregulation (Davson, 1951). Krogh (1939) has shown that an active chloride-absorbing mechanism in the gill compensates for the continual loss of the ion in the copious, dilute urine of fish. He also found that the ability to absorb chloride and the threshold of chloride concentra-

FIGURE 10.—Chloride concentrations in the serum of smallmouth bass, determined periodically from June 24 to November 18, 1958.

tion of the water from which the ion could be absorbed varied with the species.

In these experiments considerable changes were noted in the chloride concentration of the serum of the smallmouth bass. The results of the determinations on serum chloride are given in table 1 and are shown in fig. 10. After a sharp drop from 123 meq/l to 102 meq/l, serum chloride increased throughout the period July 5 to September 29, finally reaching a high of 132 meq/l on September 29. This increase was followed by a series of decreases and increases with the level at the end of the experiment being 110 meq/l. Here, as in the calcium concentration curve, there seems to be little similarity between the pattern of changes of serum chloride concentration and other constituents of the serum. Although there seems to be a cyclic nature in the behavior of this component, the timing of the cycle seems to be completely unrelated to the timing of other apparently cyclic constituents. Unfortunately, even with the amount of information available concerning the absorption and excretion of chloride, little is known about the mechanism by which these processes are controlled. The changes observed in the serum chloride concentration undoubtedly are related to the changes in the anabolic-catabolic relationship, which has been suggested as being the cause for the behavior of other components of the serum.

SERUM CHOLESTEROL

Cholesterol is an important component of living systems. It belongs to a group of crystalline alcohols known as the sterols. It apparently holds a key and central position in the metabolism of many closely related and biologically important compounds. In recent years cholesterol has come under increased scrutiny due to its possible role in cardiopathy. End products of the metabolism of cholesterol are many and include the bile acids and the varied group of steroid hormones. Almost all of the cholesterol in human serum (free and esterified) is associated with the lipoproteins (Fruton and Simmonds, 1958).

Very little information is available on cholesterol metabolism in fish. Field et al. (1943) have noted that cholesterol accounted for more than half of the total lipids found in carp blood. He also found almost one-half the cholesterol in the free state. Dombrowski (1954) reported that the cholesterol level in the blood of carp infected with carp dropsy or ascites was significantly lower than the level in healthy carp. Phillips et al. (1957) reported slightly lower values for brook trout than for brown trout.

Cholesterol levels in the serum of the smallmouth bass were subject to a great deal of variation. Figure 11 demonstrates the extent of variation encountered.

FIGURE 11.—Cholesterol concentrations in the serum of smallmouth bass, determined periodically from July 5 to November 18, 1958.

After recording three successive readings of 145 mg% from July 5 to August 2, the cholesterol level dropped more than 100 percent to a level of 65 mg% on August 18 (table 1). This drop was followed by a dramatic rise to 346 mg% on September 29. Levels greater than 300 mg% were maintained throughout the remainder of the experimental period.

Here again the metabolic patterns producing changes in serum levels of other components seemingly are at work, but unlike serum calcium and chloride, changes in serum cholesterol seem to be more directly related to variation noted in serum globulin, serum potassium, et cetera. Serum cholesterol remained low throughout the period that serum protein levels were decreasing, and reached its lowest level on August 18, which will be remembered as the date corresponding with a minimum value in serum protein, the return to apparently normal levels of nonprotein nitrogen, and maximum values in sodium, potassium, and inorganic phosphorus. The levels of serum cholesterol from the beginning of the experimental period to August 18 were extremely low compared with values obtained in other fish. Field et al. (1943) reported a range of 244 mg% to 364 mg% free cholesterol in the whole blood of carp. Phillips et al. (1957) found an average of 316 mg% cholesterol in the serum of the brook trout and 402 mg% cholesterol in the serum of the brown trout. Apparently, then, the smallmouth bass were actually in a state of hypocholesterolemia from July 5 to August 18.

In man, decreased cholesterol or hypocholesterolemia is found in conditions of hyperthyroidism, pernicious anemia, and certain types of liver disease (Hawk et al., 1954). Also, almost all of the cholesterol of human plasma is associated with lipoproteins, and these lipoproteins are identified electrophoretically with certain globulin fractions (Oncley, 1956). Here, then, are two possible explanations for the low cholesterol values:

(1) A condition of hyperthyroidism during the period July 5 to August 18 could account for the low values; or (2) it is conceivable that since cholesterol is so intimately associated with globulin that an interference in globulin metabolism might have affected serum cholesterol levels. It is apparent from figure 3 and previous discussions that globulin metabolism was interfered with during this period.

BLOOD GLUCOSE

The following discussion provides little background for the interpretation of blood glucose data in these experiments and may seem to be more detailed than is justified by available information; however, such a discussion is necessary to formulate an explanation of the possible role of energy metabolism in producing the blood composition patterns observed in the smallmouth bass. Since glucose and other carbohydrates are intimately related to energy metabolism, the discussion of carbohydrates is included in this section.

The role of blood glucose in mammalian metabolism is well known. It plays the same type role in the metabolism of fish, but certain experimental evidence suggests that its role in normal fish metabolism is more restricted than in mammals. Phillips has contributed a significant amount of work on this subject by publication of a paper on carbohydrate utilization in trout (Phillips et al., 1948). He noted that blood glucose levels of trout increased after ingestion of glucose, just as in mammals. However, the curve of the rise and fall of the blood sugar following the feeding of glucose closely resembled the curves found in studies of the diabetic man. He further noted that insulin injections quickly lowered the blood sugar after it had been raised by glucose feeding. Phil-

lips concluded that trout are unable to use urine clearance as a means of eliminating overdoses of ingested glucose when he could find no evidence of carbohydrate excretion. Feeding carbohydrates over long periods was shown to cause pathological storage of glycogen and was strongly correlated with increased mortality. On the basis of this work, Phillips suggested that the diet of trout should consist of no more than 9 percent digestible carbohydrate or that the fish should not be fed more than 4.5 grams of carbohydrate per kilogram of body weight per day.

From these experimental results it seems that fish lack the metabolic machinery to handle high levels of carbohydrate, and that this deficiency is probably due to reduced insulin supply resulting from a lack of "Islets of Langerhans" tissues; however, Strand (1958) suggested that pathological storage of glycogen could be prevented in salmon and brook trout by adding epinephrine to the diet. Epinephrine, or adrenalin, accelerates the conversion of glycogen to hexose phosphates by promoting the conversion of inactive phosphorylase to the active enzyme (Fruton and Simmonds, 1958). Apparently the inability of fish to utilize carbohydrates arises not only from a shortage of insulin, but also from the lack of a mechanism to mobilize carbohydrate reserves.

In mammals, most of the energy for metabolism is derived from the oxidation of glucose to carbon dioxide and water; but in fish this energy-producing mechanism is seriously impeded by an inability to metabolize glucose. Thus, the fish must depend on either fat or protein as a source of energy; however, oxidation of fats requires the proper functioning of the citric acid cycle. From the discussion by Haurowitz (1955) it is evident that maintenance of the citric acid cycle is dependent on carbohydrate metabolism, since efficient regeneration of oxalacetic acid in the cycle is dependent on the degradation of glucose. Further evidence of the dependency of proper fat metabolism on normal carbohydrate metabolism is shown by the appearance of symptoms of incomplete fat oxidation (ketosis) in diabetes (White et al., 1954).

From the foregoing discussion it seems likely that metabolism of fats is dependent on the metabolism of carbohydrates, and since carbohydrate metabolism in fish is inefficient, normal utilization of fats or the ability to handle high levels of dietary fat is also reduced. Evidence in support of this premise has been reported by Phillips and Podoliak (1957). Their experiments show that high dietary levels of soft fat produce edema in trout, and that high-fat diets may also cause fatty infiltration of the liver. Excessive mortalities occurred among trout with extreme fatty infiltration of the liver.

Since carbohydrate and fat metabolism are seemingly inefficient in the fish, the only other source of large amounts of energy is protein. This need for protein as a source of energy is reflected in the high levels of protein in the diet of hatchery trout. The generally accepted level of protein in trout diets is 28 percent. DeLong et al. (1958) have reported that protein levels in the diet of salmonids should be in the neighborhood of 47 percent. These values are extremely high when compared with levels in the diets of other animals. Recent work by Phillips et al. (1957) suggests that this high level of protein can be reduced by proper attention to calorie balance in the diet.

From available information, it appears that fish must utilize a higher proportion of protein for energy than must other animals. This is probably of little consequence in the normal metabolism of the fish or during a period when there is energy expenditure only for maintenance,

some growth, osmoregulation, et cetera. However, this need could have a profound effect on the metabolism of the fish during periods when a great deal of energy is required for synthesis or by an increased metabolic rate. Such needs would occur during formation of sex products, during a period of abnormally high water temperatures, or as a result of an elevated metabolic rate such as would be brought about by increased thyroid activity. In periods of abnormally high energy requirements, it is even conceivable that energy intake would not be high enough to meet the demands and actual tissue catabolism would take place.

Results of blood glucose determination are given in table 1 and are shown in figure 12. Except for one erratic value the blood glucose level increased throughout the experimental period until October 7 and decreased slightly between October 7 and November 18. Beginning at 58 mg% on June 24, the blood glucose concentration rose to 63 mg% on July 24. A rather erratic increase to 90 mg% from June 24 and August 2 was followed by a similar decrease to 68 mg% on August 18. From this point, blood glucose levels increased to 100 mg% on October 7. The level decreased slightly to 88 mg% at the end of the experimental period.

The increase in levels of blood glucose during the experimental period can possibly be explained by a decrease in rate of insulin production. This would mean that from June 24 to July 24 an abnormally high level of insulin was promoting a very rapid utilization of blood glucose, thereby keeping the glucose levels low. Fruton and Simmonds (1958) have suggested that thyroxine may stimulate insulin production. High levels of thyroxine as a result of hyperthyroid activity have already been proposed as a possible cause for the low levels of serum cholesterol noted during this period. Assuming this

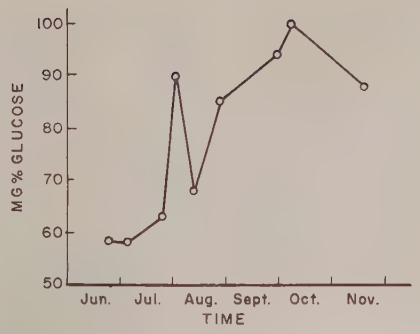

FIGURE 12.—Glucose concentrations in the blood of smallmouth bass, determined periodically from June 24 to November 18, 1958.

general hypothesis to be correct, a gradual decline in thyroid activity after July 24 with its attendant decline in the stimulation of insulin production, would result in a rise in blood sugar; however, this does not explain the rapid increase from 63 mg% to 90 mg% and the decrease to 68 mg% between July 24 and August 18.

Another possible explanation for the changes in blood glucose levels in the bass could have been a decrease in the effectiveness of insulin during this period. In mammals, the adrenal corticoids (the interrenal corticoids in fish) have been shown to be antagonistic to insulin (Long et al., 1940; Fruton and Simmonds, 1958). This effect is apparently accomplished by producing a hyperglycemia by gluconeogenesis without affecting the insulin supply directly. This increase in glucose is accompanied by an increase in nitrogen excreted, suggesting that the glucose increase is at the expense of protein. This proposal probably explains less of the observed facts in the blood glucose pattern than the hyperthyroid hypothesis, but it does possibly explain one perplexing bit of information—the erratic behavior of the glucose curve between July 24 and August 18 (fig. 12). The studies of Brodish and Long (1956) support the hypothesis that adrenal cortical trophic hor-

mone (ACTH) is released by a biphasic mechanism—a rapid release of the hormone under neural control followed by a prolonged release phase indicative of humoral regulation. If this study could be applied to fish, a biphasic release of the trophic hormone by the pituitary, bringing about a biphasic production of the interrenal corticoids, would possibly explain the biphasic increase in blood glucose. According to this proposal, the sharp rise and fall of the glucose curve from July 24 to August 18 was a result of gluconeogenesis caused by the first phase of the biphasic release of the interrenal corticoids. The more gradual rise of the glucose curve from August 18 to October 7 would correspond with the second phase of interrenal corticoid release.

BLOOD IRON

Iron exists in the body chiefly in the heme portion of the hemoglobin of the red blood cells. It is also found in plasma in combination with serum globulin and is an indispensable constituent of various oxidation-reduction enzymes.

Results of iron determinations on the blood of the smallmouth bass are given in table 1. These values demonstrate a considerable amount of variation in blood iron during the experimental period, and since most of the iron in blood is present in hemoglobin, it is apparent that hemoglobin concentrations varied considerably. Comparatively speaking, the blood of the smallmouth bass has a rather low iron content. Hall et al. (1926) reported an average value of 32.9 mg% blood iron in the menhaden. Field et al. (1943) reported an average value of 31.5 mg% blood iron (recalculated from percent hemoglobin) in the carp and an average of 25.5 mg% in the brown trout. Phillips et al. (1956) reported an average of 29.4 mg% blood iron in the brown trout. In the small mouth bass, blood iron values ranged from 12 mg% to 20 mg%, with little evidence of any systematic variation.

There is little similarity between the changes noted in blood iron and in any of the other components of the blood or serum of the smallmouth bass. Apparently, the metabolism of hemoglobin and iron is not subject to the same cyclic pattern of variation noted in the other components.

METABOLISM IN SMALLMOUTH BASS

Throughout the separate discussions in previous sections, possible explanations for experimental results obtained in this study have been proposed, but no attempt has been made to integrate these proposals. In this discussion, an attempt will be made to collect the proposed explanations into a metabolic picture.

In attempting to integrate the several discussions, the experimental results will be considered by periods.

PERIOD 1—JUNE 24 TO AUGUST 18

In the separate discussions, the following proposals have been made to explain the observed changes between June 24 and August 18:

1. Gradual decrease in serum total protein and serum globulin—caused by an increase in the catabolic rate of metabolism coupled with a decrease in the anabolic rate.

2. Increased levels of nonprotein nitrogen—resulted from an increase in protein catabolism.

3. Blood creatinine increase—muscle-tissue catabolism.

4. Lower levels of lipid phosphorus, especially between July 24 and August 2—decrease in anabolic rate.

5. Increase in serum sodium and potassium levels—increase in tissue catabolism or osmotic adjustment to compensate for loss of serum proteins.

6. Low serum cholesterol levels—hyperthyroidism or interference with globulin metabolism.

7. Relatively low glucose levels—high insulin level caused by hyperthyroidism.

It has been suggested in five of these seven components that a decrease in synthesis or anabolism coupled with an increase in destruction or catabolism would explain the data obtained in these experiments. Hyperthyroidism has been suggested as the cause of the observed results in the two other cases. The suggestion that synthesis would be reduced during this period and degradation increased seems unrealistic. During this period, water temperatures are usually nearing their summer peak and food should be readily available. It is difficult to understand how there could be a shortage of anything needed by the metabolic machinery of the smallmouth bass, at least not enough of a shortage to promote tissue catabolism.

A reduced energy supply as a result of hyperthyroid function could explain all of the observed changes described. Thyroxine, the hormone produced by the thyroid gland, uncouples oxidative phosphorylation from cellular respiration or electron transport thereby speeding up the rate at which oxidation takes place, but producing less energy in the form of ATP in the process (Maley and Lardy, 1955). Several studies on the thyroid of fish have demonstrated cyclic changes in the functioning of that gland, and several investigators have presented histological evidence of an increase in the spring of the year (Brown, 1957). Barrington and Matty (1954) found maximum thyroid activity in the minnow, *Phoxinus*, between February and May. Swift (1955) demonstrated that thyroid activity in the brown trout reached a peak in early July, and he also noted a decreased rate of growth during this period.

Peters and Van Slyke (1946) have further suggested that thyroxine causes no actual destruction of protein, but merely increases demands of the body for fuel. That fish apparently must normally utilize some protein for energy has previously been discussed, so, in hyperthyroidism, the fish would be faced with an increased demand for fuel and little other than protein to satisfy this demand. It is conceivable that the demand for protein to be used for energy could become great enough to bring about some tissue catabolism. An increase in tissue catabolism and an attendant increase in blood nonprotein nitrogen as a result of high thyroxine levels have been discussed by Peters and Van Slyke (1946).

In summary, a proposed integrated picture of metabolism for period 1 (from June 24 to August 18) would be as follows:

Increasing thyroxine levels caused by hyperthyroid function in the smallmouth bass increased the metabolic rate and at the same time reduced the amount of energy produced. With less energy available, the synthesis of serum proteins was reduced, causing a decrease in their net concentration. As a result of the increase in metabolic rate, more energy was also required. The fish could not efficiently utilize carbohydrate or fat to provide this energy, so the tissue proteins were called on to provide amino acids for energy production. As a result of this increase in tissue catabolism, nonprotein nitrogen and crea-

tinine levels in the blood increased. Lipid phosphorus, requiring less energy for synthesis, was not greatly affected during the early part of this period. With the loss of the osmotic effect of serum protein, some homeostatic device in the fish initiated an increase in serum sodium and potassium levels to maintain the number of osmotically active particles available in the plasma. Hypocholesterolemia, caused by hyperthyroidism was evident throughout this period, and an increase in insulin production brought about by high levels of blood thyroxine kept blood glucose levels low.

As the levels of circulating thyroxine increased toward the latter part of period 1, reducing even more the energy supply available for metabolism, the trends just noted would continue, but lipid phosphorus levels would also begin to show the effect of increased hyperthyroidism, finally falling rapidly between August 2 and August 18. Cholesterol levels, which have been low throughout the period, would go even lower as hyperthyroidism increased. The blood glucose concentration increased sharply from July 24 to August 2 only to return to about the July 24 level on August 18, probably as a result of the first stage in the biphasic release of corticoid hormones, proposed in the discussion of blood glucose. Sometime between July 24 and August 2, the increase in thyroid function began to abate. The levels of thyroxine were still high, but the rate of increase was not so great. By August 18, thyroxine levels had reached their highest point as was evidenced by the increase in serum protein after this time.

PERIOD 2—AUGUST 18 TO OCTOBER 7

Concentrations of serum components between August 18 and October 7 simply reflected a return to a pattern of metabolism characterized by a lower metabolic rate. Thyroxine levels were decreasing during this period and energy for synthesis was becoming readily available. Tissue catabolism was no longer needed to supply amino acids for energy production. As the protein in the plasma increased, the number of other active osmotic particles needed was reduced, so that sodium and potassium levels were lowered. Cholesterol levels freed from the influence of hyperthyroidism were beginning to rise, and glucose levels increased rapidly, probably because of the second stage in the biphasic release of gluconeogenic corticoid hormones.

PERIOD 3—OCTOBER 7 TO NOVEMBER 18

The period was characterized by a rapid increase in nonprotein nitrogen and creatinine and a moderate increase in serum lipid phosphorus and serum protein. These results would seem to be in complete disagreement with the explanation of the increase in nonprotein nitrogen, which accompanied the decrease in serum total protein during period 1.

To explain the contrasting experimental results shown in the two periods, it is necessary to compare the conditions prevailing in both periods 1 and 3. In period 1 (June 24 to August 18), food was readily available from which metabolism should have been able to produce a favorable energy balance, but due to the hyperthyroid condition, available food was utilized inefficiently, resulting in a negative energy balance and causing an increase in protein catabolism to meet energy needs. This increase in catabolism brought about an increase in serum nonprotein nitrogen and blood creatinine levels.

During period 3 (October 7 to November 18), conditions were somewhat different. By October 7, most of the fish were in the quiescent state, a physiological state

characterized by lack of activity and a state in which the fish feed very little, if at all. Also there is evidence that the thyroid of fish is less responsive to the pituitary hormone, thyrotropin, at low temperatures (Pickford and Atz, 1957). This would suggest that the metabolic rate would also be reduced somewhat. Peters and Van Slyke (1946) described elevated blood nonprotein nitrogen levels accompanied by gradually falling urinary nitrogen levels during fasting, which they suggested may be a result of accelerated nitrogen catabolism, decreased excretion, or both. Fasting accompanied by rising nonprotein nitrogen levels and low nitrogen excretion could explain the observed data here, but it is difficult to explain how serum protein synthesis could be progressing during this period of starvation.

The picture presented by nonprotein nitrogen and serum protein increase in the blood of bass in period 3 leads to interesting speculation. This rapid rate of serum protein synthesis during a period when there was no food intake suggests that the amino acids being used in that synthesis were being transported from some other tissue to the liver where the serum proteins were produced. This, then, would be selective catabolism of body protein accompanying selective anabolism, and the high levels of nonprotein nitrogen noted simply reflected a higher rate of amino acid transport for the synthesis of serum proteins.

ROLE OF CYCLIC HORMONAL RELATIONSHIPS IN BLOOD COMPOSITION CHANGES

In the reconstruction of the metabolism proposed in the previous discussions to account for changes in the blood composition of the smallmouth bass from June 24 to November 18, 1958, three general periods of different metabolic activity have been proposed as causing those changes:

(1) Period 1 (June 24 to August 18) was characterized by a reduced anabolic rate, an increased catabolic rate, and an increase in the overall metabolic rate. It was suggested that a hyperthyroid state was responsible for these conditions.

(2) A metabolic pattern in period 2 (August 18 to October 7) suggestive of a relatively lower metabolic rate was evident. During this time catabolism decreased and anabolism increased.

(3) The proposed characterization of metabolism in period 3 (October 7 to November 18) was one of selective catabolism and anabolism, a general lowering of the metabolic rate, and decreased excretion—induced by the quiescent state and the fasting condition associated with it.

In the following discussion an attempt will be made to relate the proposed changes in catabolic-anabolic balance and thyroid function to possible cyclic hormonal relationships.

Several types of cyclic physiological behavior have been noted in fish (Brown, 1957: chapters VI, VII, and IX). Among these types are sex cycles, cyclic growth-rate changes, periods of relative inactivity followed by periods of great activity, and cycles in feeding.

Environmental change has been the cause commonly suggested to explain metabolic cycles in fish, such as growth, activity, and feeding. In general, the metabolism of the fish is thought to be controlled by the environment. Thus, in cold water fish eat little and grow little. In extremely warm water they become listless and inactive. Increases in water temperature, especially from an extremely low to a more moderate level, caused increased feeding activity. Certainly, changes in environment do affect the fish in a very real manner, but apparently environment is not solely concerned. Brown

(1946) has shown that even under constant environmental conditions cyclic metabolic changes take place. In her study, 2-year-old brown trout were kept at 11.5° C. under 12 hours of artificial lighting a day, provided with a constant rate of water flow, and were allowed to eat all they would consume two times a day. She noted significant cyclic changes in growth rate of the trout several times during the experimental period of more than a year, and that they became sexually mature under these conditions. This information suggests metabolic cycles in fish that are active, even under constant environmental conditions, or that environment is not the sole cause of changes in growth rate, feeding response, and activity.

Experiments carried out by Ekberg (1957) also suggest physiological cycles in fish that act independently of environment. He studied respiration in the tissues of goldfish that had been adapted to warm and cold temperatures. In these studies, Ekberg noted that oxygen consumption by gill tissues was greater during the summer months than during the winter months, and that this relation was not affected by adaptation to warm or cold temperatures. Apparently, tissue respiration was affected more by the time of year than by changes in the environment.

In mammals the hormones are largely responsible for maintaining metabolism at a constant rate, for making the adjustments in absorption, anabolism, catabolism, excretion, et cetera, so that overall metabolism changes very little. Apparently, interrelationships among the hormones are responsible for this. For example, an increase in thyroxine in the blood speeds up the secretion of ACTH and the cortical steroids that tend to suppress thyroxine production. In turn, increasing levels of cortical steroids in the blood reduce the production of ACTH and the cortical steroids. In homotherms, the hormonal interrelationships are so closely associated with the nervous system that metabolism is finely balanced. The balance is such that a slight deviation in homeostatic function is immediately noted or evokes a response in an automatic corrective measure that is just sufficient in intensity to make the necessary adjustment. These same relationships probably exist in fish but have not reached as fine a balance, so that instead of a series of integrated checks and balances on metabolism such as those in the mammal the metabolism of the fish is controlled by a rather loosely integrated series of hormonal and neural relationships. With this type of control, hypo- or hyper-metabolic function would elicit a response by a corrective mechanism, but due to the lack of a sensitive regulating device, the corrective mechanism would over-correct. In this manner, hypothyroidism brought about by excess cortical steroid production would be followed by hyperthyroidism when the corrective relationship responded to subnormal thyroid function. This type of "teeter board" metabolism would be cyclic with respect to time.

The results of the determinations on the blood and serum of the smallmouth bass are characterized by systematic variation, a variation in which there is a trend. Such variations are apparent in 14 of the 16 components studied (see table 1). Possibly even more striking than the systematic variation noted has been the tendency for trends to reverse themselves. For example, the total serum protein levels decreased steadily for 8 weeks, then increased steadily for 12 weeks (fig. 2). These data also show an apparent relation among many of the components. Within the period August 2 to August 18, maximum or minimum values for 12 of the 16 components were recorded (figs. 2 to 12). The systematic variation, reversal of trends, and the tendency for these revers-

als to occur at about the same time in several components of the serum suggest that these changes are metabolic responses to a single physiological cause, and that this mechanism is actually responsible for the cyclic nature of the observations.

A hyperthyroid state was proposed and treated in detail in the section on Metabolism, page 27. Evidence in favor of increased thyroxine levels in the early part of the experimental period has been conclusive enough to warrant support of that idea. No attempt has been made to suggest the cause of this increase from June 24 to August 18 nor of its decrease after August 18.

Evidence has been previously presented that thyroid activity in fish increases in the spring of the year, and that this peak of activity lasts for only a few weeks (Swift, 1955; Barrington and Matty, 1954). Such cyclic activity almost certainly results from endocrine activity produced by the antagonistic effect of one hormone on another.

According to White et al. (1954), a number of gonadal steroids have been shown to affect the uptake of radioiodine, I^{131}, by the thyroid of the rat. Pickford and Atz (1957) reported the thyroid shows a lessening of activity during spawning. A period of decreased thyroid activity during spawning has been observed by Lieber (1936) in *Misgurnis fossilis*, a hibernating fish. Further evidence of the effect of spawning and sex hormones on the thyroid is presented by Hoar (1952), who noted that the mass mortality of the landlocked alewife following spawning may result from an exhausted thyroid. Summarizing extensive research on the effect of reproduction on thyroid activity, Hoar suggested that thyroid function during spawning may either be increased or diminished, depending on the life history and habits of the species (Brown, 1957).

The first blood sample in this study was taken on June 24. The time of the first sampling corresponded to the end of the spawning period of smallmouth bass in Fall Creek. At that time many abandoned nests were noted, and only a very few nests were still being guarded by the males.

It seems quite possible, then, that thyroid activity in the bass could be reduced during and immediately following the spawning period by the gonadal steroids acting as antithyroid compounds on the thyroid. Such a situation would reduce the amount of circulating thyroxine. Pickford and Atz (1957) have noted that the pituitary responds to low thyroxine levels by secreting additional amounts of thyrotropin, the thyroid activator hormone. Increased levels of thyrotropin would then lead to thyroid stimulation in the form of hypertrophy, hyperemia, and hyperplasia. All of these are conditions of hyperactivity in the thyroid; however, the synthesis of active thyroxine would still be inhibited by the sex steroids. Once spawning is completed and the level of circulating steroids has fallen (Pickford and Atz, 1957), the synthetic mechanism would be reconnected in the histologically hyperactive gland, resulting in hyperthyroidism. The resulting hyperthyroidism and its attendant reduction in energy production then could bring about the decreased anabolism and increased catabolism that have been proposed as causing a lowering of serum protein and lipid phosphorus levels, hypocholesterolemia, hypoglycemia, and as increasing the levels of sodium and potassium and nonprotein nitrogen in smallmouth bass between June 24 and August 18 of the experimental period.

No mention has been made thus far of possible causes for correction of the proposed hyperthyroid condition; however, the antagonistic effect of one hormone on another or hormonal relationships similar

to those suggested as being responsible for the condition, were probably responsible for its correction. Pickford and Atz (1957), summarizing the work of several investigators, stated that the circulating level of thyroxine undoubtedly influences thyrotropin release by the pituitary. They further noted that treatment with thyroxine or thyroid preparation causes a decrease in thyroid function as reflected in the histological picture. With this evidence in mind, it seems probable that thyroxine synthesis has been inhibited here by increased thyroxine levels or that an increase in thyroxine resulted in a decrease in the release of thyrotropin, the precursor pituitary hormone. Because of a lack of thyrotropin to stimulate the synthetic and release activities of the thyroid, once the circulating thyroxine molecules were catabolized, there were few to replace them. With this reduced replacement rate, the high serum thyroxine levels began to fall in the bass between August 2 and August 18.

There is also a possibility that still another mechanism was brought into play to correct the hyperthyroid condition. Pickford and Atz (1957) state that administration of cortical hormones (these hormones are produced by the interrenal tissue in fish) causes an inhibition of thyroid function in the intact animal. White et al. (1954) also suggest that a high level of cortical steroids may have an inhibiting influence on thyroid function. Then, in addition to high levels of thyroxine being inhibitory in nature to thyroxine synthesis and release, an increase in blood levels of the cortical steroid hormones would bring about the same result—that of lowering thyroxine levels. This is even more plausible in the light of reports by Pickford and Atz (1957) and White et al. (1954) that administration of thyroxine will bring about an increase in production and release of the pituitary hormone, adrenal cortical trophic hormone (ACTH), which in turn promotes an increase in elaboration of the cortical steroids.

Further evidence that cortical steroids may have played a part in correction of the proposed hyperthyroid state has previously been presented in the discussion of blood glucose. It was suggested there that the biphasic increase in blood glucose between July 24 and August 27 (fig. 12) was a result of a biphasic release of cortical steroids, as proposed by Brodish and Long (1956).

From the evidence presented, it is suggested that the smallmouth bass has a twofold corrective mechanism for hyperthyroidism. Hyperthyroidism tends to be reduced by the high levels of thyroxine produced by the condition and also by increased synthesis of cortical steroids which was triggered by the same high levels of thyroxine.

Mention should be made here of the possible role of the thyroid in the quiescent state of the bass. Pickford and Atz (1957) have summarized several experiments by Olivereau (1954, 1955) noting the effect of low temperature on thyroid function in trout. In Olivereau's studies, low temperature enhanced the production of thyrotropin by the pituitary in the same manner in trout as in warm-blooded animals. This increased production of thyrotropin in turn produced the histological characteristics of hyperactivity in the thyroid; however, the fixation of radioiodine, I^{131}, into the thyroxine molecule was retarded. These experiments, summarized by Pickford and Atz, suggest that low temperatures may inhibit the synthesis of thyroxine. If this were correct, the bass in the present experiments would be expected to be in a state of hypothyroidism during the quiescent period; and, in fact, hypothyroidism could be the cause of the onset of the quiescent state. Hypothyroidism in mammals is best charac-

terized by the word "slow." In hypothyroidism, the mammal is much less active, the pulse rate is low, the breathing rate is decreased, and excretion is retarded. These are also the characteristics of the quiescent smallmouth bass.

In conclusion, it should be stated that a considerable amount of work needs to be done on the phases of metabolism discussed in this paper, either to confirm or reject the hypotheses proposed. If further work demonstrates the validity of the hypotheses proposed here, and if these hypotheses are applicable to all species of fish, then future fishery research must take these proposals into account when designing experiments in which some phase of the metabolism of the fish is to be studied or is to be used to evaluate the effects of experimental conditions. The results of the tests on the blood and serum of the smallmouth bass suggest that this fish, as an experimental animal, would not be comparable in any two given periods of time within a year. For example, the smallmouth bass would not be expected to react in a similar manner to the same set of experimental conditions on June 24 and August 18. If all fish have these cyclic metabolic patterns, then in an experiment to evaluate the growth response to a new diet, a low rate of growth would not necessarily mean that the diet is deficient; instead, a low rate of growth could be a result of a hyperthyroid condition. The response of fish in tests to determine vitamin requirements might be significantly different in two periods of the year even under identical, controlled experimental conditions. These different responses might not be a result of faulty experimental technique. The difficulty in duplicating the data in the two trials might also be the result of different vitamin requirements in different phases of the cyclic metabolic patterns.

SUMMARY

The concentrations of 16 noncellular components occurring in the blood and serum of smallmouth bass (*Micropterus dolomieui*) were measured, including proteins, nonprotein nitrogen fractions, phosphorus fractions, electrolytes, and cholesterol in the serum and glucose, creatinine, and iron in the blood.

Blood samples were obtained from approximately 350 fish obtained by angling from a single stream. The blood was removed directly from the heart with a syringe.

Measurements of concentrations of the different components were made at approximately 3-week intervals from June 24 to November 18, 1958, by colorimetric chemical methods.

Concentrations of 13 of the 16 components displayed systematic rather than random variation when plotted against time.

Concentrations of globulin, total phosphorus, and lipid phosphorus in serum decreased through successive samples until August. After August the concentrations of these components increased in successive samples through the end of the experimental period in November. Serum inorganic phosphorus concentrations decreased gradually throughout the experimental period, except for a sharp increase on August 18. Serum albumin concentrations remained relatively constant until globulin returned to its initial or June 24 value, after which both globulin and albumin increased in samples through the end of the experimental period.

Total nonprotein nitrogen and blood creatinine curves were almost exact dupli-

cates with respect to shape. Concentrations of both fractions increased sharply then fell to their initial values by August 18. Concentrations of both components remained relatively constant through September, then increased in successive samples through the end of the experimental period in November.

Serum sodium and potassium levels tended to behave in an opposite manner to levels of the globulin and phosphorus fractions. The levels of these cations increased until August 18 and decreased afterward. Serum calcium concentrations, however, decreased in successive samples through August 27, then increased through the end of the experimental period.

Serum cholesterol levels remained constant in samples through August 2, dropped sharply on August 18, then increased to roughly two times the initial value by the end of the experimental period. Blood glucose concentrations, in general, increased throughout the experimental period. Serum uric acid, serum chloride, and blood iron concentrations behaved in somewhat cyclic manner, but not to the extent of the components previously discussed.

Blood samples taken on August 18 showed highest concentrations reached by uric acid, inorganic phosphorus, sodium, and potassium, and lowest concentrations of nonprotein nitrogen, creatinine, total protein, globulin, and cholesterol.

Available data suggest that changes in the anabolic-catabolic balance were responsible for the cyclic concentrations of the various components and that the most likely explanation of these changes is in the changes in levels of circulating thyroxine.

Antagonistic effects of the sex hormones during spawning and of circulating thyroxine on the thyroid gland are suggested as possible causes for the changes in levels of circulating thyroxine.

It is suggested that the antagonistic effect of one hormone on the production of another hormone produces a total metabolism in the smallmouth bass that would be cyclic in nature when plotted against time.

If metabolism in fish is cyclic with respect to time, the norm can be determined only if the time of year the blood samples were collected is included in the data. This suggests that in experiments in which some phase of metabolism is used to measure effects of a treatment the results (response of the fish) are dependent on time of year the experiment is performed. Such results would be applicable only when the metabolic rates of the experimental fish and of the fish from which the data came are of the same magnitude.

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